epilepsy with Rolandic-sylvian onset seizures. In addition, the effects of ESM on ENM and atypical absences suggest the involvement of thalamo-cortical circuitry in the epileptic network. ABPE is a unique age-related epilepsy involving the Rolandic-sylvian plus thalamo-cortical networks in the developing brain of children.

Disclosure of conflicts of interest

The authors have no financial or personal relations that could pose a conflict of interest.

Acknowledgment

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.braindev.2012.12.011.

References

[1] Aicardi J, Chevrie JJ. Atypical benign partial epilepsy of childhood. Dev Med Child Neurol 1982;24:281–92.

[2] Beaumanoir A, Ballis T, Varfis G, Ansari K. Benign epilepsy of childhood with Rolandic spikes. A clinical, electroencephalographic, and telencephalographic study. Epilepsia 1974;15:301–15.

[3] Bernardina BD, Tassinari CA. EEG of a nocturnal seizure in a patient with "benign epilepsy of childhood with Rolandic spikes". Epilepsia 1975;16:497–501.

[4] Blom S, Heijbel J. Benign epilepsy of children with centro-temporal EEG foci. Discharge rate during sleep. Epilepsia 1975;16:133–40.

[5] Fejerman N, Caraballo R, Tenembaum SN. Atypical evolutions of benign localization-related epilepsies in children: are they predictable? Epilepsia 2000;41:380–90.

[6] Oguni H, Uehara T, Tanaka T, Sunahara M, Hara M, Osawa M. Dramatic effect of ethosuximide on epileptic negative myoclonus: implications for the neurophysiological mechanism. Neuropediatrics 1998;29:29–34.

[7] Kubota M, Nakura M, Hirose H, Kimura I, Sakakihara Y. A magnetoencephalographic study of negative myoclonus in a patient with atypical benign partial epilepsy. Seizure: J Br Epilepsy Assoc 2005;14:28–32.

[8] Minami T, Tasaki K, Yamamoto T, Gondo K, Yanai S, Ueda K. Magneto-encephalographical analysis of focal cortical heterotopia. Dev Med Child Neurol 1996;38:945–9.

[9] Ishitobi M, Nakasato N, Yamamoto K, Iinuma K. Opercular to interhemispheric source distribution of benign Rolandic spikes of childhood. NeuroImage 2005;25:417–23.

[10] RamachandranNair R, Otsubo H, Shroff MM, Ochi A, Weiss SK, Rutka JT, et al. MEG predicts outcome following surgery for intractable epilepsy in children with normal or nonfocal MRI findings. Epilepsia 2007;48:149–57.

[11] Dale AM, Liu AK, Fischl BR, Buckner RL, Belliveau JW, Lewine JD, et al. Dynamic statistical parametric mapping: combining fMRI and MEG for high-resolution imaging of cortical activity. Neuron 2000;26:55–67.

[12] Shiraishi H, Ahlfors SP, Stufflebeam SM, Takano K, Okajima M, Knake S, et al. Application of magnetoencephalography in epilepsy patients with widespread spike or slow-wave activity. Epilepsia 2005;46:1264–72.

[13] Sakurai K, Tanaka N, Kamada K, Takeuchi F, Takeda Y, Koyama T. Magnetoencephalographic studies of focal epileptic activity in three patients with epilepsy suggestive of Lennox–Gastaut syndrome. Epileptic Disord 2007;9:158–63.

[14] Kelemen A, Barsi P, Gyorsok Z, Sarac J, Szucs A, Halasz P. Thalamic lesion and epilepsy with generalized seizures, ESES and spike-wave paroxysms – report of three cases. Seizure: J Br Epilepsy Assoc 2006;15:454–8.

[15] Battaglia D, Veggiotti P, Lettori D, Tamburrini G, Tartaglione T, Graziano A, et al. Functional hemispherectomy in children with epilepsy and CSWS due to unilateral early brain injury including thalamus: sudden recovery of CSWS. Epilepsy Res 2009;87:290–8.

[16] Guzzetta F, Battaglia D, Veredice C, Donvito V, Pane M, Lettori D, et al. Early thalamic injury associated with epilepsy and continuous spike-wave during slow sleep. Epilepsia 2005;46:889–900.

[17] Hanaya R, Sasa M, Ujihara H, Fujita Y, Amano T, Matsubayashi H, et al. Effect of antiepileptic drugs on absence-like seizures in the tremor rat. Epilepsia 1995;36:938–42.

[18] Marescaux C, Micheletti G, Vergnes M, Depaulis A, Rumbach L, Warter JM. A model of chronic spontaneous petit mal-like seizures in the rat: comparison with pentylenetetrazol-induced seizures. Epilepsia 1984;25:326–31.

[19] van Rijn CM, Sun MS, Deckers CL, Edelbroek PM, Keyser A, Renier W, et al. Effects of the combination of valproate and ethosuximide on spike wave discharges in WAG/Rij rats. Epilepsy Res 2004;59:181–9.

[20] Kobayashi K, Nishibayashi N, Ohtsuka Y, Oka E, Ohtahara S. Epilepsy with electrical status epilepticus during slow sleep and secondary bilateral synchrony. Epilepsia 1994;35:1097–103.

[21] Tassinari CA, Rubboli G, Volpi L, Meletti S, D'Orsi G, Franca M, et al. Encephalopathy with electrical status epilepticus during slow sleep or ESES syndrome including the acquired aphasia. Clinical Neurophysiol 2000;111(Suppl. 2):S94–S102.

[22] Patry G, Lyagoubi S, Tassinari CA. Subclinical "electrical status epilepticus" induced by sleep in children. A clinical and electroencephalographic study of six cases. Arch Neurol 1971;24:242–52.

Phenotypic Spectrum of *COL4A1* Mutations: Porencephaly to Schizencephaly

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Objective: Recently, *COL4A1* mutations have been reported in porencephaly and other cerebral vascular diseases, often associated with ocular, renal, and muscular features. In this study, we aimed to clarify the phenotypic spectrum and incidence of *COL4A1* mutations.

Methods: We screened for *COL4A1* mutations in 61 patients with porencephaly and 10 patients with schizencephaly, which may be similarly caused by disturbed vascular supply leading to cerebral degeneration, but can be distinguished depending on time of insult.

Results: *COL4A1* mutations were identified in 15 patients (21%, 10 mutations in porencephaly and 5 mutations in schizencephaly), who showed a variety of associated findings, including intracranial calcification, focal cortical dysplasia, pontocerebellar atrophy, ocular abnormalities, myopathy, elevated serum creatine kinase levels, and hemolytic anemia. Mutations include 10 missense, a nonsense, a frameshift, and 3 splice site mutations. Five mutations were confirmed as de novo events. One mutation was cosegregated with familial porencephaly, and 2

mutations were inherited from asymptomatic parents. Aberrant splicing was demonstrated by reverse transcriptase polymerase chain reaction analyses in 2 patients with splice site mutations.

Interpretation: Our study first confirmed that *COL4A1* mutations are associated with schizencephaly and hemolytic anemia. Based on the finding that *COL4A1* mutations were frequent in patients with porencephaly and schizencephaly, genetic testing for *COL4A1* should be considered for children with these conditions.

Type IV collagens are basement membrane proteins that are expressed in all tissues, including the vasculature. COL4A1 ($\alpha 1$ chain) and COL4A2 ($\alpha 2$ chain) are the most abundant type IV collagens, and form heterotrimers with a 2:1 stoichiometry ($\alpha 1 \alpha 1 \alpha 2$).[1] Mutations in *COL4A1* and *COL4A2* cause sporadic and hereditary porencephaly, a neurological disorder characterized by fluid-filled cysts in the brain that often cause hemiplegia or tetraplegia.[2–4] In addition, a variety of clinical phenotypes, including small vessel disease affecting the brain, eyes, and kidneys, are associated with *COL4A1* abnormality[5,6]: neonatal porencephaly and adult stroke,[7] sporadic extensive bilateral porencephaly resembling hydranencephaly,[8] periventricular leukomalacia with intracranial calcification,[9] HANAC (hereditary angiopathy with nephropathy, aneurysm, and muscle cramps) syndrome,[10,11] Axenfeld–Rieger anomaly with leukoencephalopathy, and adult stroke and intracerebral hemorrhage.[12–14] Notably, *COL4A1* mutations were present in 2 patients with muscle–eye–brain/Walker–Warburg syndrome (MEB/WWS), which is characterized by ocular dysgenesis, neuronal migration defects, and congenital muscular dystrophy, suggesting that *COL4A1* is also involved in normal cortical and muscular development in humans.[15] Consistent with this hypothesis, a mouse model of a heterozygous *COL4A1* mutation (*Col4a1*[+/Δex40]) showed ocular dysgenesis, cortical neuronal localization defects, and myopathy, along with cerebral hemorrhage and porencephaly.[2,15] The phenotypic spectrum of *COL4A1* mutations is expanding; however, the whole spectrum of systemic phenotypes and the incidence of *COL4A1* mutations associated with porencephaly has not been systemically examined.

In this study, we screened for *COL4A1* mutations in 61 patients with porencephaly and 10 patients with schizencephaly, which may be similarly caused by disturbed vascular supply leading to cerebral degeneration, but can be distinguished depending on time of insult.[2–4,16,17] *COL4A1* mutations were identified in 10 patients with porencephaly and 5 patients with schizencephaly, who showed a variety of associated findings, including intracranial calcification, focal cortical dysplasia (FCD), ocular abnormalities, pontocerebellar atrophy, myopathy, elevated serum creatine kinase levels, and hemolytic anemia. Our study demonstrated the importance of genetic testing for *COL4A1* in children with porencephaly or schizencephaly.

Patients and Methods

Patients

A total of 61 patients with porencephaly including a previous cohort with porencephaly,[4] and 10 patients with schizencephaly including a patient who also had porencephaly were analyzed for *COL4A1* mutations. Schizencephaly is defined as transmantle clefts bordered by polymicrogyria in adjacent cortex.[18] The clefts extended through the entire hemisphere, from the ependymal lining of the lateral ventricles to the pial covering of the cortex.[19] The clefts are further divided into those with closed lips and those with open lips. In the clefts with closed lips, the walls affix each other directly, obliterating the cerebrospinal fluid space within the cleft at that point.[20] *COL4A2* mutations were negative for these patients. Genomic DNA was isolated from blood leukocytes according to standard methods, and amplified using an illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare, Buckinghamshire, UK). The DNA of familial members of patient 6 was isolated from saliva samples using Oragene (DNA Genotek, Kanata, Ontario, Canada). Experimental protocols were approved by the committee for ethical issues at Yokohama City University School of Medicine. All patients were investigated in agreement with the requirements of Japanese regulations.

Mutation Analysis

Exons 1 to 52, covering the entire *COL4A1* coding region, were examined by high-resolution melting (HRM) curve analysis. Samples showing an aberrant melting curve pattern in the HRM analysis were sequenced. Polymerase chain reaction (PCR) primers and conditions are shown in Supplementary Table S1. All novel mutations were verified using original genomic DNA, and screened in 200 Japanese normal controls by HRM analysis. For the family showing de novo mutations, parentage was confirmed by microsatellite analysis, as previously described.[21] Biological parents were confirmed if >4 informative markers were compatible and other markers showed no discrepancy.

Reverse Transcriptase-PCR

Reverse transcriptase (RT)-PCR using total RNA extracted from lymphoblastoid cell lines (LCL) was performed essentially as previously described.[22] Briefly, total RNA was extracted using RNeasy Plus MiniKit (Qiagen, Tokyo, Japan) from LCL with or without $30 \mu M$ cycloheximide (CHX; Sigma, Tokyo, Japan) incubation for 4 hours. Four micrograms total RNA was subjected to reverse transcription, and $2 \mu l$ cDNA was used for PCR. Primer sequences are ex20-F (5′-CCCAAAAGGTTTCC CAGGACTACCA-3′) and ex22-R (5′-GTCCGGGCTGACAT TCCACAATTC-3′; for patient 4); and ex22-F (5′-GAATTC CAGGGCAGCCAGGATTTAT-3′) and ex24-R (5′-CATCTCT GCCAGGCAAACCTCTGT-3′; for patient 7). DNA of each

PCR band was purified by QIAEXII Gel extraction kit (Qiagen; for patient 4) and E.Z.N.A. poly-Gel DNA Extraction kit (Omega Bio-Tek, Norcross, GA; for patient 7), respectively.

Results

Mutation and RT-PCR analysis

COL4A1 abnormalities were identified in 15 patients (Fig 1 and Table). Nine mutations occurred at highly conserved Gly residues in the Gly-X-Y repeat of the collagen triple helical domain. Interestingly, a missense mutation (c.4843G>A [p.Glu1615Lys]) at an evolutionary conserved amino acid and a nonsense mutation (c.4887C>A [p.Tyr1629X]) were found in the carboxy-terminal noncollagenous (NC1) domain. The other 4 mutations include a frameshift mutation (c.2931dupT [p.Gly978TrpfsX15]) and 3 splice site mutations (c.1121-2dupA, c.1382-1G>C, and c.1990+1G>A). None of these mutations was present in 200 Japanese normal controls, and Web-based prediction tools suggested that these mutations are pathogenic (Supplementary Table S2). The c.2842G>A (patient 1), c.3976G>A (patient 2), c.4887C>A (patient 8), c.2689G>A (patient 13), and c.1990+1G>A (patient 14) mutations occurred de novo. The c.3995G>A mutation (patient 3) was not found in the mother's DNA (the father's DNA was unavailable). The c.1121-2dupA (patient 4) and c.2931dupT (patient 6) mutations were found in the asymptomatic fathers. c.1963G>A (patient 10) was found in familial members affected with porencephaly as well as asymptomatic carriers, suggesting incomplete penetrance of the mutation (Supplementary Fig S1). The remaining patients' parental DNA was unavailable.

To examine the mutational effects of the 2 splice acceptor site mutations (c.1121-2dupA and c.1382-1G>C), RT-PCR and sequencing were performed (see Fig 1). c.1121-2dupA caused the deletion of exon 21 from the wild-type COL4A1 mRNA, resulting in an in-frame 55-amino acid deletion (p.Gly374_Asn429delinsAsp). The effect of c.1382-1G>C was more complicated. There were 3 PCR products amplified from LCL treated with CHX, which inhibits nonsense-mediated mRNA decay (NMD). The middle band corresponded to the wild-type allele. The sequence of the lower mutant band showed a 33bp insertion of intron 22 and an 84bp deletion of all of exon 23 from the use of cryptic splice acceptor and donor sites within intron 22. The change of amino acid sequence from this mutant transcript was a deletion of 29 amino acids and an insertion of 12 amino acids (p.Gly461_Gly489delinsValHisCysGlyAsp-PheTrpSerHisValThrArg). The upper band was only observed in CHX-treated LCL, but was not evident in

the untreated LCL, suggesting that this mutant transcript may undergo NMD. Sequencing of the upper band showed a 61bp insertion of intron 22 from the use of a cryptic splice acceptor site within intron 22, as mentioned above. The product of this mutant transcript leads to a frameshift, creating a premature stop codon (p.Gly461ValfsX31), which is consistent with degradation of the mutant transcript by NMD.

Clinical Features

The clinical information for individuals with COL4A1 mutations is summarized in the Table, and their representative brain images are shown in Figure 2 and Supplementary Fig S2. COL4A1 mutations were identified in 10 of 61 patients with porencephaly (16.4%). Of note, COL4A1 mutations were identified in 5 of 10 patients with schizencephaly (50.0%), revealing a novel association between COL4A1 mutations and schizencephaly. Thirteen patients were born at term, and 2 patients (patients 1 and 12) were born at preterm. Their body weight was normal at birth except for 5 patients (patients 3, 4, 9, 12, and 15) who were below −2.0 standard deviations. The occipitofrontal circumference was available in 12 patients, and 6 patients (patients 2, 3, 6, 13, 14, and 15) were below −2.0 standard deviations. Two patients (patients 11 and 12) were confirmed to have an antenatal hemorrhage as previously reported.[23,24] Among associated findings with COL4A1 mutations, a patient showed FCD that was histologically demonstrated (Fig 3A–F). In addition, hemolytic anemia was found in 5 of 15 patients, suggesting that hemolytic anemia may be a novel feature associated with COL4A1 mutation. Ponto-cerebellar atrophy along with severe bilateral porencephaly was observed in 2 patients, and a patient showed cerebellar hypoplasia. Previously reported magnetic resonance imaging and systemic findings associated with COL4A1 mutations were also observed, including intracranial calcification (7 of 15), myopathy (1 of 15; see Fig 3G, H), ocular abnormalities (4 of 15), and elevated serum creatine kinase levels (6 of 15), confirming that these features are useful signs for COL4A1 testing. Case reports are available in the Supplementary Data.

Discussion

We found a total of 15 novel mutations in this study. Nine mutations occurred at highly conserved Gly residues in the Gly-X-Y repeat of the collagen triple helical domain, suggesting that these mutations may alter the collagen IV α1α1α2 heterotrimers.[1,25] We reported for the first time 2 mutations (a nonsense and a missense change) in the NC1 domain. The nonsense mutation

A

Patient 6
c.2931dupT
p.Gly978TrpfsX15

Patient 8
c.4887C>A
p.Tyr1629X

7S
domain

Collagen triple
helical domain

NC1
domain

1   1669

Patient 5	Patient 10	Patient 11	Patient 13	Patient 1	Patient 15	Patient 12	Patient 2	Patient 3	Patient 9
c.1835G>A	c.1963G>A	c.2608G>A	c.2689G>A	c.2842G>A	c.3122G>A	c.3245G>A	c.3976G>A	c.3995G>A	c.4843G>A
p.Gly612Asp	p.Gly655Arg	p.Gly870Arg	p.Gly897Ser	p.Gly948Ser	p.Gly1041Glu	p.Gly1082Glu	p.Gly1326Arg	p.Gly1332Asp	p.Glu1615Lys

H. Sapiens
M. musculus
G. gallus
X. tropicalis
T. rubripes
C. elegans

B

ag ex21

WT   g t t g c a g G C
Mutant t t g c a a g G C

c.1121-2dupA

C

RT(+)

Patient 4   Father   Mother   RT(-)

bp
700—
500—
400—
300—

D

ex20-F

ex22-R

Upper band
(WT allele)

36 bp   165 bp   96 bp

ex20   cag   ex21   ex22

Ex20   Ex21   Ex21   Ex22

C C T C C A G G C C T C C C   T A C A C A A T G G A A T

Lower band
(Mutant allele)

ex20   caag   ex21   ex22

Ex20   Ex22

C C T C C A G A T G G A A T

E

ag ex23

WT   t t t a g G T C A
Mutant t t t a c G T C A

c.1382-1G>C

F

Patient 7

untreated   CHX treated
Control RT(+) RT(-)   RT(+) RT(-)

bp
300—
200—
75—

G

ex22-F

ex24-R

Upper band
(Mutant allele 1)

96 bp   2048 bp   61 bp   84 bp   545 bp   71 bp

ex22   gt   ag   ex23   gt   ag   ex24

exon 22   intron 22   intron 22   exon 23

G A G A A A G tg c a c tg   t t t t t a g GT C A A A A

Middle band
(WT allele)

2109 bp

ex22   gt   ag   gt   ag   ex23   gt   ag   ex24

exon 22   exon 23   exon 23   exon 24

G A G A A A G G T C A A A A   G A A A T A G G T T T C C C

Lower band
(Mutant allele 2)

33 bp   28 bp

ex22   gt   ag   gt   ag   ex23   gt   ag   ex24

exon 22   intron 22   intron 22   exon 24

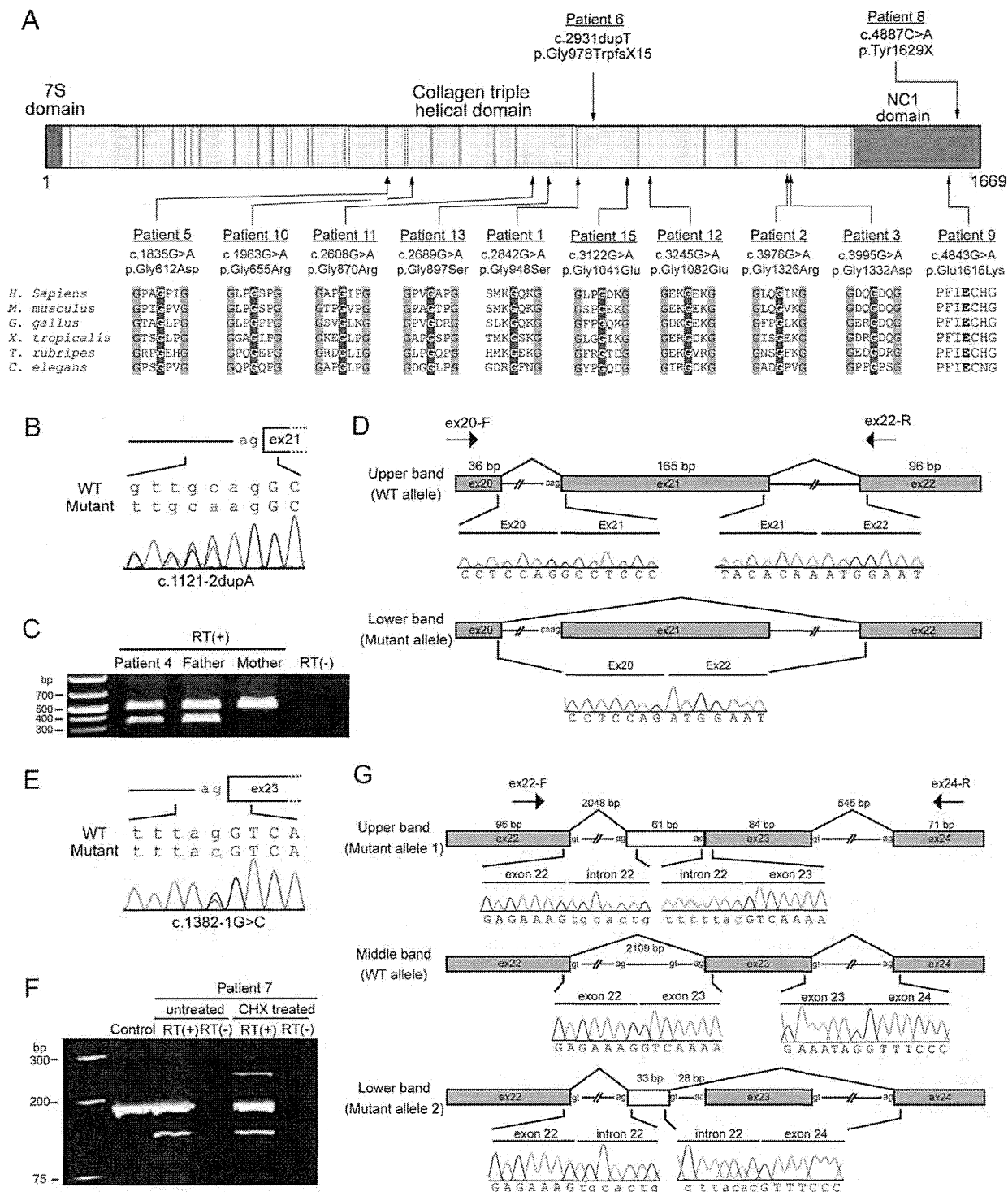G A G A A A G tg c a c tg   g t t a c a c G T T T C C C

FIGURE 1: *COL4A1* mutations in patients with porencephaly or schizencephaly. (A) Functional domains of COL4A1 protein. The locations of 12 mutations, including 10 missense mutations (bottom), a nonsense mutation, and a frameshift mutation (top) are indicated by arrows. The 7S domain is highlighted with blue and the NC1 domain with red. Gly-X-Y repeats within the collagen triple helical domain are highlighted with yellow. All of the missense mutations occurred at evolutionary conserved amino acids. The positions of the conserved Gly residues in the Gly-X-Y repeats are highlighted in gray. Homologous sequences were aligned using CLUSTALW (http://www.genome.jp/tools/clustalw/). (B) The c.1121-2dupA mutation in intron 20 is colored red. Sequences of exons and introns are presented in upper and lower cases, respectively. (C) Reverse transcriptase (RT)-polymerase chain reaction (PCR) analysis of patient 4 and his parents. (D) Schematic presentation of the wild-type (WT; upper) and mutant (lower) transcripts and primers used for analysis. A single band (500bp), corresponding to the WT allele, was amplified using the mother's cDNA template. Conversely, a lower band was detected from the cDNA from the patient and his father. In the mutant transcript, the 165bp exon 21 was deleted. Sequences of exons and introns are presented in upper and lower cases, respectively. (E) The c.1382-1G>C mutation in intron 22 is colored red. (F) RT-PCR analysis of patient 7 and a control. (G) Schematic representation of the WT and mutant transcripts, and primers used for analysis. A single band (183bp), corresponding to the WT allele, was amplified using a control cDNA template. Conversely, upper and lower bands were detected from the patient's cDNA. The upper band (244bp), which was observed only in cycloheximide (CHX)-treated cells, had a 61bp insertion of intron 22 sequences, leading to a frameshift. Absence of the upper band in untreated lymphoblastoid cell lines strongly suggests that the mutant transcript may undergo nonsense-mediated mRNA decay. The lower band had a 33bp insertion of intron 22 and 84bp deletion of the whole of exon 23, leading to an in-frame 51bp deletion.

TABLE: Clinical features of patients with *COL4A1* mutations

Cases	Age	Sex	Mutation	Inheritance	Brain MRI/ CT findings	CP	Epi	Ocular features	Family history	ID	Hyper-CK	Other
1	14y	M	c.2842G>A (p.Gly948Ser)	de novo	Bilateral POCE, calcification, hemosiderin deposition	Q	+	-	-	+	-	
2	18m	M	c.3976G>A (p.Gly1326Arg)	de novo	Bilateral SCZ, calcification, hemosiderin deposition	Q	+	-	-	+	-	
3	15m	M	c.3995G>A (p.Gly1332Asp)	Absent in mother	Unilateral SCZ, calcification, hemosiderin deposition	H	+	-	-	+	-	
4	6y	M	c.1121-2dupA[1]	Paternal	Unilateral POCE	H	+	-	-	+	-	FCD
5	2m	F	c.1835G>A (p.Gly612Asp)	ND	Bilateral SCZ, calcification, thin CC, thin brain stem, cerebellar atrophy, absence of SP, hemosiderin deposition, multicystic encephalomalacia,	Q	+	Optic nerve hypoplasia	-	+	+	HA
6	7y	M	c.2931dupT (p.Gly978TrpfsX15)	Paternal	Unilateral POCE	H	+	-	-	+	+	
7	12y	F	c.1382-1G>C[2]	ND	Unilateral POCE	H	+	-	-	+	+	Myopathy
8	10y	M	c.4887C>A (p.Tyr1629X)	de novo	Unilateral POCE	H	+	-	Hematuria	+	-	
9	3m	F	c.4843G>A (p.Glu1615Lys)	ND	Bilateral POCE, calcification, hypoplastic CC, hemosiderin deposition, thin	Q	+	Microphthalmia Corneal opacity	-	+	-	VSD, HA

TABLE (Continued)

Cases	Age	Sex	Mutation	Inheritance	Brain MRI/ CT findings	CP	Epi	Ocular features	Family history	ID	Hyper-CK	Other
					brain stem, cerebellar atrophy, multicystic encephalomalacia							
10	2y7m	F	c.1963G>A (p.Gly655Arg)	Paternal[3]	Bilateral POCE	Q	+	-	POCE, Epi	+	-	-
11	1y	F	c.2608G>A (p.Gly870Arg)	ND	Unilateral POCE, calcification	Q	+	Congenital cataract	-	+	-	-
12	1y5m	M	c.3245G>A (p.Gly1082Glu)	ND	Unilateral SCZ with bilateral POCE, calcification, cerebellar hypoplasia	T	+	Congenital cataract	-	+	-	HA, Hematuria
13	3y7m	M	c.2689G>A (p.Gly897Ser)	de novo	Unilateral POCE	Q	+	-	-	+	+	-
14	9m	F	c.1990+1G>A	de novo	Unilateral POCE, hemosiderin deposition	Q	+	-	-	+	+	HA, Hematuria
15	2y	F	c.3122G>A (p.Gly1041Glu)	ND	Unilateral SCZ, hemosiderin deposition	Q	+	-	-	+	+	HA

1) p.Gly374_Asn429delinsAsp change was predicted by mRNA analysis
2) Two alternative protein chages were predicted by mRNA analysis: p.Gly461_Gly489delinsValHisCysGlyAspPheTrpSerHisValThrArg and p.Gly461ValfsX31. y, years; m, months; M, male; F, female; ND, Not determined; POCE, porencephaly, SCZ, schizencephaly; CC, corpus callosum; SP, septum pellucidum; CP, cerebral palsy; H, hemiplegia; T, Triplegia; Q, quadriplegia; Epi, epilepsy; ID, intellectual disability; CK, creatine kinase; FCD, Focal cortical dysplasia; HA, Hemolytic anemia; VSD, ventricular septal defect
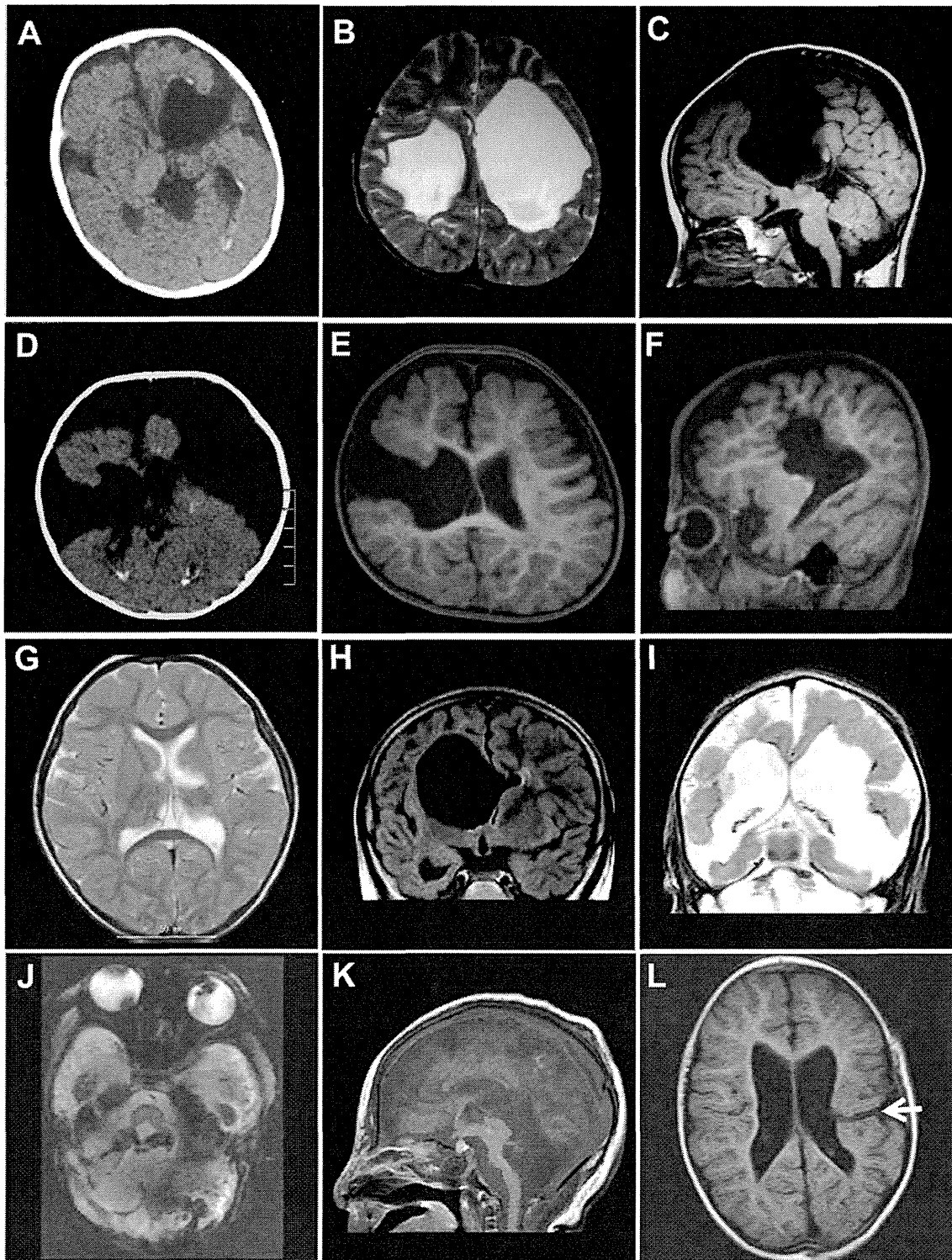3) Co-segregation of the p.Gly655Arg mutation with porencephaly was confirmed.

FIGURE 2: Computed tomography (CT) scan (A, D) and magnetic resonance imaging (MRI; B, C, E–L) of patients with *COL4A1* muta-tions. (A–C) Images of patient 1. (A) The CT scan shows calcification along with the dilated lateral ventricular wall. (B) T2-weighted and (C) T1-weighted images (WIs) at 5 years of age showing bilateral porencephaly. (D) The CT image of patient 2 with schizence-phaly shows calcification of the lateral ventricular wall and brain parenchyma. (E, F) T1-WIs of patient 3 show unilateral schizence-phaly at 15 months of age. (G) T2-WI of patient 4 at 3 years of age shows parenchymal defect of the left thalamus and basal ganglia due to subependymal hemorrhage. (H) Fluid-attenuated inversion recovery image of patient 7 at 6 years of age showing unilateral porencephaly. (I) T2-WI, (J) T2*-weighted gradient-echo image (WGRE), and (K) T1-WI of patient 9. (I) The MRI at 2 months of age shows bilateral porencephaly with low-intensity lesions along with a deformed ventricular wall, which has hemosiderin deposition and calcification. (J) T2*-WGRE showing hemosiderin deposition in the atrophic cerebellum. The atrophic pontocerebellar structures are also shown in (K). (L) T1-WI of patient 15 showed schizencephaly in the left hemisphere at 2 years of age.
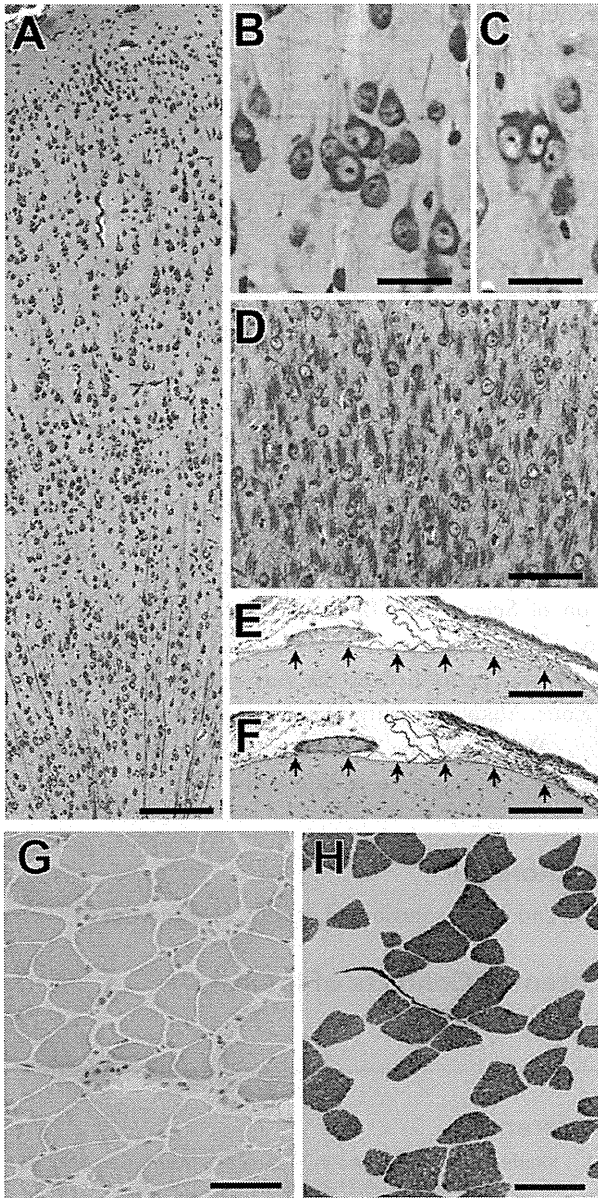
FIGURE 3: Histopathological features of the resected frontal tissue of patient 4 (A–F) and biopsied rectus abdominis muscle of patient 7 (G, H). (A) Low-magnification view of the cortex showing architectural abnormalities. (B, C) Two examples of neuronal clustering. (D) Many neurons scattered within the subcortical white matter. (E, F) Two serial sections demonstrating the superficial layer of the cortex. Note that the basal lamina of the pia mater (arrows in each panel) is continuously labeled with antibodies against collagen type IV (E) and laminin (F). (A–D) Klüver–Barrera stain. (E, F) Immunostained and then counterstained with hematoxylin. (G) Hematoxylin and eosin staining showing variation in fiber size, slightly increased endomysial connective tissue, and internal nuclei. (H) Adenosine triphosphatase (pH 4.5) staining showing type 2B fiber deficiency. There was no increase in number of type 2C fibers. Scale bars indicate 175μm (A, E, F), 30μm (B, C), 80μm (D), and 30μm (G, H).

would cause a truncation of the NC1 domain rather than mRNA degradation by NMD as the mutation was located within 50bp of the exon–intron boundary of the

second to last exon (exon 51).[26] The NC1 domains are the sites for molecular recognition through which the stoichiometry of chains in the assembly of triple-helical formation is directed[1]; therefore, these 2 mutations may alter the assembly of the collagen IV α1α1α2 heterotrimers. In addition, the effect of 2 splice site mutations was examined using LCL, suggesting that in-frame deletion/insertion mutant protein should be produced. Thus, it is highly likely that impairment of the collagen IV α1α1α2 heterotrimer assembly caused by mutant α1 chain is a common pathological mechanism of COL4A1 mutations. The c.2931dupT mutation found in patient 6 and his father might cause severe truncation of COL4A1 protein. It is possible that the truncation of COL4A1 protein can also impair α1α1α2 heterotrimer assembly similar to substitutions of conserved Gly residues in the Gly-X-Y repeat. Alternatively, the mutant transcript might undergo NMD, and haploinsufficiency of COL4A1 might cause a weakness of basement membrane. Biological analysis using patients' cells will clarify these possibilities.

COL4A1 mutations in schizencephaly were first demonstrated in this study. Schizencephaly was used by Yakovlev and Wadsworth in 1946 to describe true clefts formed in the brain as a result of failure of development of the cortical mantle in the zones of cleavage of the primary cerebral fissures.[19] Schizencephaly is differentiated from clefts in the central mantle that arise as the result of a destruction of the cerebral tissues, which they called encephalocrastic porencephalies, now known simply as porencephaly.[19] Schizencephaly has been understood as a neuronal migration disorder, because the clefts are lined by abnormal gray matter, described as polymicrogyria. Conversely, porencephaly is understood to be a postmigration accident resulting in lesions, without gray matter lining the clefts or an associated malformation of cortical development. It has been suggested that both schizencephaly and porencephaly are caused by encephaloclastic regions, and can be distinguished depending on time of insult.[16,17] The present study clearly demonstrated that COL4A1 mutations caused both porencephaly and schizencephaly, supporting the same pathological mechanism for these 2 conditions.

The genes responsible for FCD have been elusive, despite extensive investigation. The pathological features of the cortical tubers of tuberous sclerosis (TSC) may be indistinguishable from those of FCD. Apart from FCD due to TSC, there is only 1 gene that may explain the genetic basis of FCD, where a homozygous mutation in CNTNAP2 has been identified in Amish children with FCD, macrocephaly, and intractable seizures.[27] Surprisingly, the present study discovered a patient with FCD

and porencephaly, in whom aberrant splicing was demonstrated and FCD1A was pathologically confirmed using resected brain tissues. A recent report revealed *COL4A1* mutations in 2 patients with MEB/WWS showing cobblestone lissencephaly,[15] and abnormal cortical development has been observed in mouse models of *COL4A1* mutations.[15,28] Thus, it is possible that *COL4A1* mutations are involved in cerebral cortical malformations, including FCD. Identification of a greater number of cases is required to confirm the association between *COL4A1* mutations and cortical malformations in humans.

In a few children, the sequelae were much more severe than would be expected on the basis of their imaging findings. This is of importance when counseling parents with regard to prediction of neurodevelopmental outcome.

Two patients with *COL4A1* mutations showed intracranial calcification, pontocerebellar atrophy, ocular abnormalities, and hemolytic anemia associated with severe bilateral porencephaly (patient 9) or schizencephaly (patient 5). Severe hemorrhagic destructive lesions in the cerebrum were observed in these patients, and T2* images also showed hemorrhage in the cerebellum, which may have resulted in a thin brainstem and severe cerebellar atrophy. Thus, these 2 patients could be considered as the most severe manifestations affecting the developing brain and eyes. A common feature of the 2 patients is hemolytic anemia of an unknown cause, which required frequent blood transfusions. Five of 15 patients with *COL4A1* mutations showed hemolytic anemia. Interestingly, 2 reports have demonstrated that mouse *Col4a1* mutants showed a significant reduction in red blood cell (RBC) number and hematocrit.[28,29] Given that *Col4a1* mutations lead to hemorrhage, chronic hemorrhage is possibly involved in RBC loss. Alternatively, the *Col4a1* mutation may directly affect blood progenitor cells, as they transmigrate across basement membranes before entering the peripheral blood.[30] Hemolytic anemia in patients with *COL4A1* mutations would imply the latter explanation. Further studies are required to clarify how *COL4A1/Col4a1* mutations are involved in anemia.

In summary, we found 15 mutations in *COL4A1* among 71 patients with porencephaly or schizencephaly, showing an unexpectedly high percentage of mutations (about 21%) in these patients. Fourteen patients with *COL4A1* mutations had no family history of cerebral palsy. The 15 patients with *COL4A1* mutations showed a variety of phenotypes, further expanding the possible clinical spectrum of *COL4A1* mutations to include schizencephaly, FCD, pontocerebellar atrophy, and hemolytic anemia. Genetic testing for *COL4A1* should be recommended for children with porencephaly and schizencephaly.

Authorship

Y.Y. and K.H. contributed equally to this work.

Potential Conflicts of Interest

Nothing to report.

References

1. Khoshnoodi J, Pedchenko V, Hudson BG. Mammalian collagen IV. Microsc Res Tech 2008;71:357–370.

2. Gould DB, Phalan FC, Breedveld GJ, et al. Mutations in Col4a1 cause perinatal cerebral hemorrhage and porencephaly. Science 2005;308:1167–1171.

3. Breedveld G, de Coo IF, Lequin MH, et al. Novel mutations in three families confirm a major role of COL4A1 in hereditary porencephaly. J Med Genet 2006;43:490–495.

4. Yoneda Y, Haginoya K, Arai H, et al. De novo and inherited mutations in COL4A2, encoding the type IV collagen alpha2 chain cause porencephaly. Am J Hum Genet 2012;90:86–90.

5. Vahedi K, Alamowitch S. Clinical spectrum of type IV collagen (COL4A1) mutations: a novel genetic multisystem disease. Curr Opin Neurol 2011;24:63–68.

6. Lanfranconi S, Markus HS. COL4A1 mutations as a monogenic cause of cerebral small vessel disease: a systematic review. Stroke 2010;41:e513–e518.

7. van der Knaap MS, Smit LM, Barkhof F, et al. Neonatal porencephaly and adult stroke related to mutations in collagen IV A1. Ann Neurol 2006;59:504–511.

8. Meuwissen ME, de Vries LS, Verbeek HA, et al. Sporadic COL4A1 mutations with extensive prenatal porencephaly resembling hydranencephaly. Neurology 2011;76:844–846.

9. Livingston J, Doherty D, Orcesi S, et al. COL4A1 Mutations associated with a characteristic pattern of intracranial calcification. Neuropediatrics 2011;42:227–233.

10. Plaisier E, Gribouval O, Alamowitch S, et al. COL4A1 mutations and hereditary angiopathy, nephropathy, aneurysms, and muscle cramps. N Engl J Med 2007;357:2687–2695.

11. Alamowitch S, Plaisier E, Favrole P, et al. Cerebrovascular disease related to COL4A1 mutations in HANAC syndrome. Neurology 2009;73:1873–1882.

12. Coutts SB, Matysiak-Scholze U, Kohlhase J, Innes AM. Intracerebral hemorrhage in a young man. CMAJ 2011;183:E61–E64.

13. Sibon I, Coupry I, Menegon P, et al. COL4A1 mutation in Axenfeld-Rieger anomaly with leukoencephalopathy and stroke. Ann Neurol 2007;62:177–184.

14. Weng YC, Sonni A, Labelle-Dumais C, et al. COL4A1 mutations in patients with sporadic late-onset intracerebral hemorrhage. Ann Neurol 2012;71:470–477.

15. Labelle-Dumais C, Dilworth DJ, Harrington EP, et al. COL4A1 mutations cause ocular dysgenesis, neuronal localization defects, and myopathy in mice and Walker-Warburg syndrome in humans. PLoS Genet 2011;7:e1002062.

16. Friede R. Porencephaly, hydranencephaly, multicystic encephalopathy. In: Developmental neuropathology. 2nd ed. Berlin, Germany: Springer-Verlag, 1989:28–43.

17. Govaert P. Prenatal stroke. Semin Fetal Neonatal Med 2009;14: 250–266.

18. Barkovich AJ, Guerrini R, Kuzniecky RI, et al. A developmental and genetic classification for malformations of cortical development: update 2012. Brain 2012;135:1348–1369.

19. Yakovlev PI, Wadsworth RC. Schizencephalies; a study of the congenital clefts in the cerebral mantle; clefts with fused lips. J Neuropathol Exp Neurol 1946;5:116–130.

20. Barkovich AJ, Kjos BO. Schizencephaly: correlation of clinical findings with MR characteristics. AJNR Am J Neuroradiol 1992;13: 85–94.

21. Saitsu H, Kato M, Mizuguchi T, et al. De novo mutations in the gene encoding STXBP1 (MUNC18–1) cause early infantile epileptic encephalopathy. Nat Genet 2008;40:782–788.

22. Saitsu H, Kato M, Okada I, et al. STXBP1 mutations in early infantile epileptic encephalopathy with suppression-burst pattern. Epilepsia 2010;51:2397–2405.

23. Lichtenbelt KD, Pistorius LR, De Tollenaer SM, et al. Prenatal genetic confirmation of a COL4A1 mutation presenting with sonographic fetal intracranial hemorrhage. Ultrasound Obstet Gynecol 2012;39:726–727.

24. de Vries LS, Koopman C, Groenendaal F, et al. COL4A1 mutation in two preterm siblings with antenatal onset of parenchymal hemorrhage. Ann Neurol 2009;65:12–18.

25. Engel J, Prockop DJ. The zipper-like folding of collagen triple helices and the effects of mutations that disrupt the zipper. Annu Rev Biophys Biophys Chem 1991;20:137–152.

26. Nagy E, Maquat LE. A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. Trends Biochem Sci 1998;23:198–199.

27. Strauss KA, Puffenberger EG, Huentelman MJ, et al. Recessive symptomatic focal epilepsy and mutant contactin-associated protein-like 2. N Engl J Med 2006;354:1370–1377.

28. Favor J, Gloeckner CJ, Janik D, et al. Type IV procollagen missense mutations associated with defects of the eye, vascular stability, the brain, kidney function and embryonic or postnatal viability in the mouse, Mus musculus: an extension of the Col4a1 allelic series and the identification of the first two Col4a2 mutant alleles. Genetics 2007;175:725–736.

29. Van Agtmael T, Bailey MA, Schlotzer-Schrehardt U, et al. Col4a1 mutation in mice causes defects in vascular function and low blood pressure associated with reduced red blood cell volume. Hum Mol Genet 2010;19:1119–1128.

30. Janowska-Wieczorek A, Marquez LA, Nabholtz JM, et al. Growth factors and cytokines upregulate gelatinase expression in bone marrow CD34(+) cells and their transmigration through reconstituted basement membrane. Blood 1999;93:3379–3390

脳形成異常と遺伝子

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Brain Malformations and Genetic Factors

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　　Historically, brain malformations have been classified based on a postmortem examination. The advancement and spread of neuroimaging techniques, particularly magnetic resonance imaging, has made it easier to identify many types of brain malformations, but it has also made classify them more complicated. Moreover, the unveiling of the genes responsible for brain malformations has dramatically changed the classification scheme itself and now most doctors have trouble following it. Although the relationship between genotype and phenotype is complicated, it can be divided into two types, locus heterogeneity and pleiotropy. One of the representative diseases demonstrating locus heterogeneity is holoprosencephaly, which shows an identical disorder of forebrain cleavage caused by 14 genes, such as *SHH* and *ZIC2*, involved in a ventrodorsal patterning of the early prosencephalon. The *ARX* gene shows a typical pleiotropic effect and its mutation causes a wide range of developmental disturbances ranging from severe brain malformations, such as hydranencephaly and lissencephaly, to nonmalformative forms of epileptic encephalopathies, such as Ohtahara syndrome and West syndrome, dyskinetic cerebral palsy, and nonsyndromic mental retardation with a strong genotype-phenotype correlation. Accurate diagnosis based on the most recent knowledge is critical for precise prediction as well as genetic counseling.

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はじめに

　画像診断技術の進歩と普及により生前から多くの脳形成異常が確認され，一部は外科的な治療対象になっている．その一方，遺伝子解析手法の進歩により脳形成異常の原因遺伝子が加速度的に同定され，脳形成異常の分類根拠は，歴史的に病理から画像，そして原因遺伝子へと軸足を移している．病変が片側性もしくは局在性で遺伝性要素は少ないと考えられていた疾患でも原因遺伝子が明らかにされ始めている．

　本稿では，原因遺伝子同定と分子病態の解明が盛んな脳形成異常について最近の知見を紹介したい．

脳形成異常の分類

　脳形成異常の分類は，1980 年代以降 MRI の開発と普及によって多種多数の脳形成異常が生前から診断されるようになり，病理分類のみでは区分されないものが増えてきた．また，1990 年代以降には脳形成異常の原因遺伝子が次々に同定され，脳の発生に関する分子生物学的な新しい知見が集積されるようになった[5]．従来の滑脳症 I 型と II 型のような一見類似の形態異常でも，分子病態

Table 1 Classification profile for malformations of cortical development (modified from ref. 1)

Ⅰ. **Malformations secondary to abnormal neuronal and glial proliferation or apoptosis**
  A. Severe congenital microcephaly
  B. Megalencephaly
  C. Cortical dysgenesis with abnormal cell proliferation but without neoplasia
    (hemimegalencephaly, focal cortical dysplasias [FCD] typeⅡ, and tuberous sclerosis)
  D. Cortical dysgenesis with abnormal cell proliferation and neoplasia
    (DNET, ganglioglioma)

Ⅱ. **Malformations due to abnormal neuronal migration**
  A. Malformations with neuroependymal abnormalities
    (periventricular heterotopia)
  B. Malformations due to generalized abnormal transmantle migration
    (lissencephaly and subcortical band heterotopia)
  C. Malformations presumably due to localized abnormal late radial or tangential transmantle migration
  D. Malformations due to abnormal terminal migration and defects in pial limiting membrane
    (Walker-Warburg syndrome, muscle-eye-brain disease, Fukuyama congenital muscular dystrophy, and congenital muscular dystrophy with cerebellar hypoplasia)

Ⅲ. **Malformations due to abnormal postmigrational development**
  A. Malformations with polymicrogyria (PMG) or cortical malformations resembling PMG
    (schizencephaly)
  B. Cortical dysgenesis secondary to inborn errors of metabolism
    (mitochondrial and pyruvate metabolic disorders, peroxisomal disorders)
  C. Focal cortical dysplasias (without dysmorphic neurons, FCD typesⅠ, Ⅲ)
  D. Postmigrational developmental microcephaly

はまったく異なることが明らかになり，現在は，病理所見に加え，主に MRI 所見と脳の発生機序に基づき分類されている（Table 1）[1]．原因遺伝子同定と分子病態解明は継続途上であり，数年ごとに分類が更新され複雑性を増しているが，正確な診断は診療においても併発症と予後の予測や遺伝相談に必須であり，最新の分類を知る必要がある．

遺伝型と表現型の相関関係

遺伝子解析技術の進歩に伴い，脳形成異常原因遺伝子が多数同定され，原因遺伝子と脳形成異常の関連性が明らかになってきた．神経細胞移動異常症に限ってみても両者の関連性は交絡し，一見複雑であるが（Fig. 1），遺伝子と遺伝子発現の結果として観察される表現型の関係は，座位異質性（locus heterogeneity）と多面発現（pleiotropy）の 2 つに区分される．座位異質性とは，染色体上の遺伝子座位が異なる複数の遺伝子によって同一の表現型が認められる現象であり，座位異質性が強い場合は表現型による原因遺伝子の推定が困難になる．多面発現とは，一つの遺伝子によって一見無関係な複数の表現型をきたすことであり，同じ遺伝子の変異でも症状に変動が
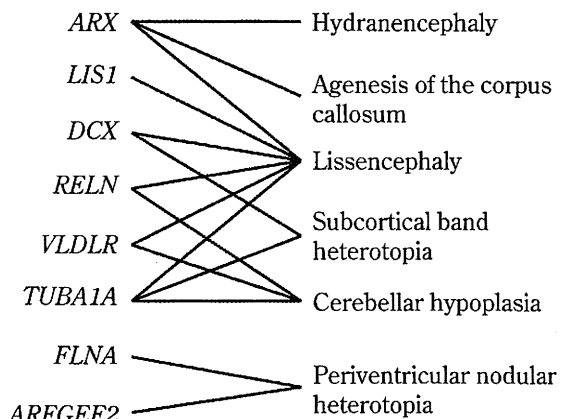

Fig. 1 Causative genes responsible for neuronal migration disorders
The left side shows the gene symbols linked with the associated disorders on the right side. Note that both *ARX* and *TUBA1A* show pleiotropy and that lissencephaly shows locus heterogeneity.

生じる．

座位異質性を示す代表的な脳形成異常として全前脳胞症が挙げられる（Fig. 2）．全前脳胞症は，左右の大脳半球を形成する前脳の腹側誘導が障害されて神経管がそのまま膨らみ，左右の分離が障害された状態である[11]．13
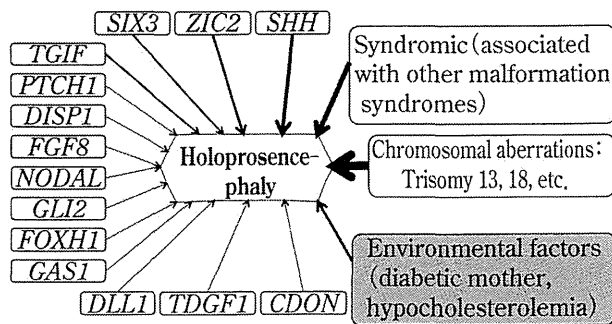
Fig. 2 Locus heterogeneity in holoprosencephaly

Holoprosencephaly is a distinct disease with different etiologies. Each arrow's size indicates the relative frequency of its etiologies.
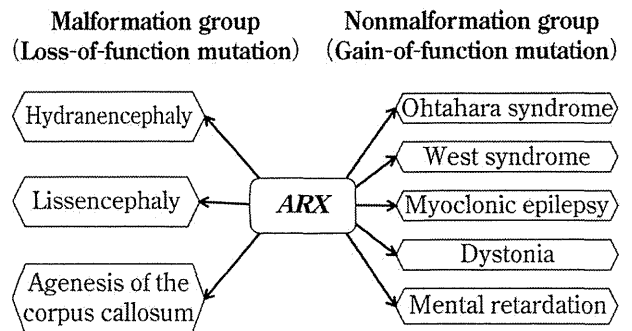

Fig. 3 Pleiotropic mutations in *ARX*: "Interneuronopathies"

Mutations of the *ARX* gene cause malformative or nonmalformative disorders. The malformation group is caused by the loss-of-function mutation, while the nonmalformation group is caused by the gain-of-function mutation. Both of these disorders are designated as interneuronopathies in view of the fact that the *ARX* gene is crucial to the development of GABAergic interneurons of the forebrain.

トリソミーや 18 トリソミーに併発するほか，コレステロール代謝異常を病態とする Smith-Lemli-Opitz 症候群に併発しやすく，母体糖尿病など環境要因も原因となり得る．家族性では単一遺伝子異常が多く，ソニックヘッジホッグ（*SHH*）[8]，*ZIC2* など前脳の腹側誘導に関与する遺伝子の変異によって全前脳胞症が引き起こされる[2]．

多面発現を示す代表的な遺伝子は，γ アミノ酪酸（GABA）作動性大脳介在ニューロンの発生に関与する *ARX* 遺伝子である（**Fig. 3**）．大脳皮質の介在ニューロンは内側基底核原基で産生され，皮質に対して接線方向に移動（tangential migration）し，皮質直下の脳室帯から放射状に移動（radial migration）する投射ニューロンとともに皮質層を形成する．ナンセンス変異やフレームシフト変異など ARX タンパク質が産生されなくなる機能喪失変異ではヒトでもマウスでも大脳介在ニューロンが欠損し，ヒトでは外性器異常を伴う X 連鎖性滑脳症もしくは水無脳症（XLAG）をきたす[10]．XLAG はヒトではじめて tangential migration の異常が明らかにされた疾患であり，ヘミ接合性の男児では後頭優位の滑脳症（約70％は前頭が厚脳回，後頭が無脳回の grade 3）と脳梁欠損に加え，小陰茎，停留睾丸など外性器の低形成を示す[3]．症状は非常に重篤であり，抑制系の破綻によって興奮性が高まり，全例で生後 24 時間以内にミオクローヌスを主とする難治性の痙攣を生じ，約半数は生後 1 年以内に死亡する[6]．ARX 機能喪失変異の女性保因者では，約半数が脳梁欠損を示す．一方，ARX のポリアラニン配列の伸長変異や C 末端のアリスタレスドメインを破壊する変異は，ARX の本来の機能である転写抑制効果を高める機能獲得変異であり，脳形成異常を伴わない大田原症候群や West 症候群などのてんかん性脳症のほか，ジ

ストニアや非症候性精神遅滞の原因となる[4)7)9)]．ARX 遺伝子の遺伝型と表現型は強い相関関係を示し，広範な表現型も介在ニューロンの発生障害に起因すると考えられ，「介在ニューロン病（interneuronopathies）」としての概念に包含される[6)12)]．

おわりに

脳形成異常だけでなく，腫瘍遺伝学や薬理遺伝学などの進歩もめざましい．次世代シークエンサーの出現はこの流れをさらに加速し，発症リスクや周術期管理，治療法選択，予後予測に個人の遺伝情報が臨床応用され，日常診療においても遺伝学の知識が求められる時代が目の前に迫っている．欧米では染色体 G バンド法よりも精度の高いマイクロアレイ検査が遺伝検査の第一選択になっているにもかかわらず，日本の保険制度の下では認められず，遺伝子診断も保険適応が限定され，諸外国との遺伝診療の差は広がりつつある．研究進歩の著しい脳形成異常の診療に際しては特に最新の知識が必要である．筆者の施設では包括的な脳形成異常の画像診断と遺伝子解析を行っており，ホームページ（http://www.id.yamagata-u.ac.jp/Ped/medical/neurology_top.html）をご参照いただければ幸いである．

文 献

1) Barkovich AJ, Guerrini R, Kuzniecky RI, Jackson GD, Dobyns WB：A developmental and genetic classification

for malformations of cortical development: update 2012. *Brain* **135**: 1348-1369, 2012.

2) Dubourg C, David V, Gropman A, Mercier S, Muenke M, Odent S, Pineda-Alvarez DE, Roessler E: Clinical utility gene card for: Holoprosencephaly. *Eur J Hum Genet* **19**: preceeding 118-120, 2011.

3) Kato M, Das S, Petras K, Kitamura K, Morohashi K, Abuelo DN, Barr M, Bonneau D, Brady AF, Carpenter NJ, Cipero KL, Frisone F, Fukuda T, Guerrini R, Iida E, Itoh M, Lewanda AF, Nanba Y, Oka A, Proud VK, Saugier-Veber P, Schelley SL, Selicorni A, Shaner R, Silengo M, Stewart F, Sugiyama N, Toyama J, Toutain A, Vargas AL, Yanazawa M, Zackai EH, Dobyns WB: Mutations of *ARX* are associated with striking pleiotropy and consistent genotype-phenotype correlation. *Hum Mutat* **23**: 147-159, 2004.

4) Kato M, Das S, Petras K, Sawaishi Y, Dobyns WB: Polyalanine expansion of *ARX* associated with cryptogenic West syndrome. *Neurology* **61**: 267-276, 2003.

5) Kato M, Dobyns WB: Lissencephaly and the molecular basis of neuronal migration. *Hum Mol Genet* **12** Spec No 1: R89-96, 2003.

6) Kato M, Dobyns WB: X-linked lissencephaly with abnormal genitalia as a tangential migration disorder causing intractable epilepsy: proposal for a new term, "interneuronopathy". *J Child Neurol* **20**: 392-397, 2005.

7) Kato M, Koyama N, Ohta M, Miura K, Hayasaka K: Frameshift mutations of the *ARX* gene in familial Ohtahara syndrome. *Epilepsia* **51**: 1679-1684, 2010.

8) Kato M, Nanba E, Akaboshi S, Shiihara T, Ito A, Honma T, Tsuburaya K, Hayasaka K: Sonic hedgehog signal peptide mutation in a patient with holoprosencephaly. *Ann Neurol* **47**: 514-516, 2000.

9) Kato M, Saitoh S, Kamei A, Shiraishi H, Ueda Y, Akasaka M, Tohyama J, Akasaka N, Hayasaka K: A longer polyalanine expansion mutation in the *ARX* gene causes early infantile epileptic encephalopathy with suppression-burst pattern (Ohtahara syndrome). *Am J Hum Genet* **81**: 361-366, 2007.

10) Kitamura K, Yanazawa M, Sugiyama N, Miura H, Iizuka-Kogo A, Kusaka M, Omichi K, Suzuki R, Kato-Fukui Y, Kamiirisa K, Matsuo M, Kamijo S, Kasahara M, Yoshioka H, Ogata T, Fukuda T, Kondo I, Kato M, Dobyns WB, Yokoyama M, Morohashi K: Mutation of *ARX* causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat Genet* **32**: 359-369, 2002.

11) Muenke M: Holoprosencephaly Overview. in: *GeneReviews*. (http://www.ncbi.nlm.nih.gov/books/NBK1530/)

12) Quille ML, Carat S, Quemener-Redon S, Hirchaud E, Baron D, Benech C, Guihot J, Placet M, Mignen O, Ferec C, Houlgatte R, Friocourt G: High-throughput analysis of promoter occupancy reveals new targets for *Arx*, a gene mutated in mental retardation and interneuronopathies. *PLoS One* **6**: e25181, 2011.

―――― 要　旨 ――――

脳形成異常と遺伝子

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　脳形成異常の分類は，原因遺伝子と脳発生機序に基づいて数年ごとに更新されている．原因となる遺伝型と表現型の関連性は複雑だが，座位異質性と多面発現の 2 つに分けられる．座位異質性を示す代表が全前脳胞症であり，*SHH*, *ZIC2* など前脳腹側化に関与する多様な遺伝子の変異によって同一の表現型が現れる．一方，多面発現の代表が *ARX* 遺伝子であり，機能喪失変異は脳形成異常をきたすが，機能獲得変異は脳形成異常を伴わない精神遅滞やてんかんなど幅広い多様な表現型を示す．脳形成異常の遺伝子同定は進歩が著しく，最新の情報に基づいた診療が望ましい．

―――― 脳外誌　22：252-255，2013 ――――

4．遺伝子変異による先天異常

2）滑脳症（神経細胞移動異常症）

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KEY WORDS　神経細胞移動異常症，滑脳症，異所性灰白質，多小脳回，
Miller-Dieker 症候群

はじめに

　滑脳症 lissencephaly は，脳回の幅が広くなり平滑な大脳表面を有する疾患であり，胎生6〜20週に脳室の近くで増殖した神経細胞が皮質に移動する過程の障害で生じる神経細胞移動異常症のひとつである[1]。以前は福山型先天性筋ジストロフィーなどに併発する脳形成異常もⅡ型滑脳症と呼ばれていたが，現在は丸石様皮質異形成 cobblestone cortical dysplasia として分離されている。狭義の滑脳症は古典型滑脳症 classical lissencephaly を意味し，脳葉がほぼ全体的に一つの脳回で形成される無脳回 agyria と，脳回の幅は広いが複数個の脳溝によって脳葉が明瞭に区切られる厚脳回 pachygyria が形態的な特徴である（図1）。軟膜から白質境界までの皮質の厚さは通常2〜3 mm だが，皮質の厚さ，すなわち皮質の6層構造は神経細胞の正常に制御された精緻な移動によって形成されており，神経細胞の移動が乱れている滑脳症では無脳回で10 mm 以上，厚脳回で4〜9 mm

に厚くなり，顕微鏡的にも大脳皮質の正常な6層構造が失われている[2)3)]。その他の神経細胞移動異常症として，皮質下帯状異所性灰白質や脳室周囲結節状異所性灰白質，多小脳回が挙げられる。それぞれの疾患は，特徴的な画像所見と臨床的特徴を示し，遺伝子解析技術の進歩により原因も数多く解明されており，最新の研究成果に基づいた正確な情報提供が求められる。本稿では，滑脳症を代表とする神経細胞移動異常症について，鑑別に役立つ疾患ごとの画像的特徴と分子病態からみた形成機序，臨床症状の違いと日常診療における注意点について述べる。

Ⅰ．古典型滑脳症単独群（無脳回・厚脳回・皮質下帯状異所性灰白質）

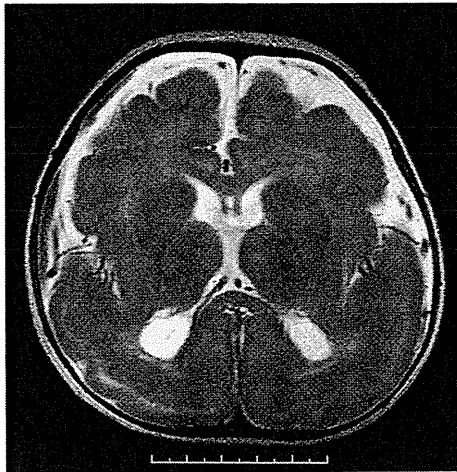　古典型滑脳症単独群は，病変の主体が大脳に限局し，上述した無脳回と厚脳回を特徴とする一群である。神経細胞の移動が皮質に到達せずに白質内で停止し，皮質下白質内に一塊の神経細胞集団を形成する皮質下帯状異所

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図1 古典型滑脳症グレード3
頭が厚脳回, 後頭が無脳回

図2 皮質下帯状異所性灰白質
後頭優位の皮質直下に皮質と等信号の異所性灰白質を連続性に認める.

性灰白質（図2）も，無脳回や厚脳回と原因遺伝子が同じであり，無脳回・厚脳回・皮質下帯状異所性灰白質は連続体としてとらえられている[2]。臨床症状の重症度は画像の重症度に比例し，大脳全体が無脳回を示し脳溝が全く認められない最重症のグレード1から脳溝が浅い程度で脳回の幅は正常だが皮質下に帯状異所性灰白質を認める軽症のグレード6まで6段階に分けられる[4]。無脳回が主体のグレード1とグレード2では，乳児期から低緊張によるフロッピーインファントとウエスト症候群（点頭てんかん）を呈し，脳波ではヒプスアリスミア以外に広汎性の高振幅速波が特徴的である。てんかん発作は難治であり，幼児期以降の精神運動発達も重症例が多い。皮質下帯状異所性灰白質が主体のグレード6では運動障害は稀である。てんかん発作が主症状であり，全般発作，部分発作の出現頻度はほぼ同じである。無脳回と異なり，ウエスト症候群の頻度は10％未満と少ない。知能予後は形態異常の程度とてんかん発作の程度に左右され，正常から重度までさまざまである。
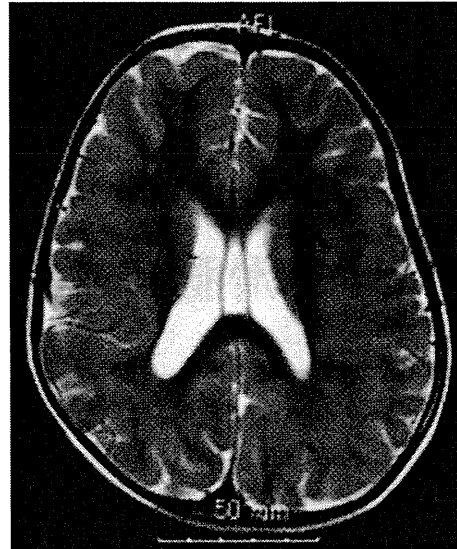
原因の多くは*LIS1*もしくは*DCX*遺伝子変異である。細胞移動には移動方向に突起を

伸展後，細胞内の核を突起伸展方向に移動させる必要がある。LIS1とDCXは核の移動に関与する微小管の関連タンパク質であり，変異による機能異常によって細胞移動が阻害される[1]。両遺伝子ともに無脳回から皮質下帯状異所性灰白質までさまざまな程度の形態異常を示し，大脳の前後方向で重症度を見比べた場合，*LIS1*変異の場合は前頭に比べ後頭がより重度であり，*DCX*変異の場合は後頭に比べ前頭がより重度である。完全な無脳回と全周性の皮質下帯状異所性灰白質では前頭優位か後頭優位か判断が付かない場合もあるが，グレード2からグレード5までは頭部MRIの所見で*LIS1*変異か*DCX*変異かを鑑別できる。*LIS1*変異の場合は，常染色体優性遺伝で浸透率が100％なので遺伝性はほぼ否定される。FISH法で17番染色体の*LIS1*の欠失を確認するとともに，親が保因者となりうる均衡型転座を除外するためにG-band法を用いた染色体検査が必要である。また，欠失などの*LIS1*変異でも生殖細胞のモザイク変異がありえるので，遺伝子診断は正確な遺伝相談が可能な施設に紹介することが望ま

しい。一方、*DCX* 変異の場合は、X連鎖性であり遺伝性が問題となる。*DCX* 変異により男児では無脳回を、女児では皮質下帯状異所性灰白質を来し、母が保因者の可能性があるので、遺伝相談が必要である。

II．Miller-Dieker 症候群

Miller-Dieker 症候群は、8の字形を呈するグレード1の無脳回に、広い前額や側頭部の陥凹、耳介低位、小さく短い鼻、上向きの鼻孔、薄い口唇などの特異顔貌と内臓奇形を伴う症候群である。重度の精神運動発達遅滞を呈し、筋緊張低下、難治性のてんかん、摂食障害のほか、肺炎を繰り返し、生命予後は不良である。全例 *LIS1* と *YWHAE* を含む17p13.3領域の欠失による隣接遺伝子症候群であり、多くは FISH 法により診断される。切断点が *LIS1* 遺伝子内に存在する場合は、*LIS1* の部分欠失となり FISH 法で検出できないこともあるが、その場合でも MLPA 法により診断可能である。

III．外性器異常を伴うX連鎖性滑脳症 X-linked lissencephaly with abnormal genitalia（XLAG）

XLAG は後頭優位の古典型滑脳症に加え、脳梁欠損と基底核異常、白質障害を示す非常に重篤な疾患である（**図3**）。生直後からミオクローヌスを主体とする難治性のけいれん発作を来し、その後も慢性難治性の下痢や低血糖、体温調節障害、呼吸障害により約半数は生後1年以内に死亡する。全例染色体は46, XY だが、小陰茎、停留睾丸、尿道下裂から女性型まで外性器の低形成を示す。生後24時間以内にけいれんを来し、CT で脳形成異常が疑われる症例では XLAG の可能性を念頭に、性別判断は慎重に行う必要がある。滑脳症は後頭優位であり、多くは前頭が厚脳回、後頭が無脳回のグレード3である。脳梁

図3　外性器異常を伴うX連鎖性滑脳症
前頭が厚脳回、後頭が無脳回のグレードグレードの古典型滑脳症に加え、脳梁欠損と基底核の異常を認める.

欠損による後角優位の脳室拡大（体脳症）を伴い、皮質の厚さは薄い場合もあり、重症例では後頭で大脳が欠損した水無脳症を呈する。女性保因者の約半数は脳梁欠損を示す。

原因遺伝子の *ARX* は転写因子としてγアミノ酪酸（GABA）作用性大脳介在ニューロンの発生を調節する。XLAG や水無脳症は機能が喪失するナル変異で生じ、*ARX* 内のポリアラニン配列の伸長変異では脳奇形を伴わない精神遅滞やジストニア、点頭てんかん、大田原症候群を来す。いずれも抑制系の異常が病態と考えられ「介在ニューロン病」とよばれる[5]。微小管の構成タンパク質であるチュブリンをコードする *TUBA1A* の変異でも脳梁欠損を併発する古典型滑脳症を来す。*TUBA1A* 変異では外性器は正常であり、橋小脳低形成を伴うことが多いので、臨床的に鑑別が可能である。

IV．脳室周囲結節状異所性灰白質 periventricular nodular heterotopia（PNH）
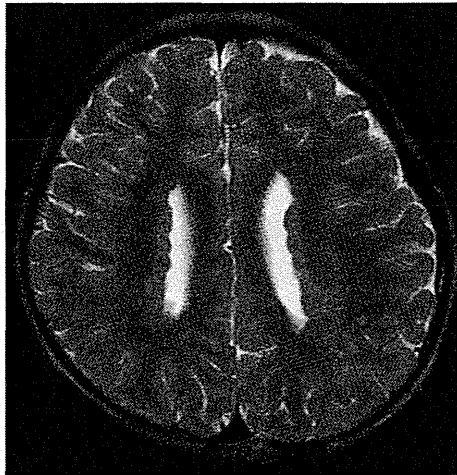
PNH は、脳回には異常がないが、一部の神経細胞が増殖部位である脳室帯もしくは脳

図4 脳室周囲結節状異所性灰白質
脳回形成は正常だが，側脳室壁に沿って凹凸のある
異所性灰白質を認める．

図5 多小脳回
両側のシルビウス裂が開大し，島から続く前頭葉の
一部で，脳回は不規則になり皮質は肥厚している．

室下帯でそのまま移動を停止し，側脳室の外
側壁に沿って灰白質を認める（図4）。てん
かん発作が主症状であり，運動障害は伴わず
知的発達も多くは正常であり，男性の胎生致
死による女性発症の家族例が多い。両側の前
角から体部側脳室壁に結節状の異所性灰白質
を認める典型例では約半数で *FLNA* 変異が
原因であり，その多くは女性である[6]。
FLNA はアクチン結合タンパク質であるフ
ィラミンAをコードし，*FLNA* 変異により
神経細胞が脳表側へ移動する際に起きる多極
性細胞から双極性細胞への変化が阻害され，
移動が障害される。フィラミンAはアクチン
以外のさまざまなタンパク質とも結合し，
PNH に Ehlers-Danlos 症候群や血管異常を
伴う例や，PNH とは別に骨格異常や弁膜症，
腸閉塞症状など多彩な病像を来す。

V．多小脳回 polymicrogyria

多小脳回は，病理学的に異常に薄く構築の
乱れた皮質とたくさんの小さい融合した脳回
を示す。皮髄境界が鮮明な新生児期には
MRI でも文字通り薄く小さい脳回が多数集
まって認められる場合もあるが，乳児期後半
以降はこのような所見が認められることは少

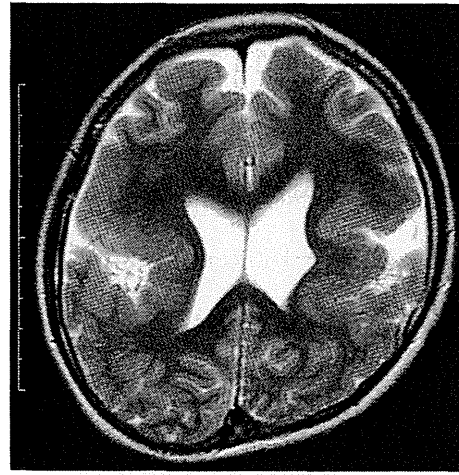なくなり，浅い脳溝で区切られた大小不規則
な幅の脳回と不規則だが一見厚い皮質を呈す
ることが多い。厚脳回や丸石様皮質異形成と
画像では鑑別が難しい場合もあるが，半数以
上の症例は中心溝とシルビウス裂の周辺に病
変が局在する両側傍シルビウス裂多小脳回で
ある[7]（図5）。症状は病変分布に比例し，
約半数の症例にてんかん発作を認めるほか，
偽性球麻痺と口腔機能障害による構語，咀
嚼，嚥下の障害が特徴的である。神経細胞移
動異常症の中で最も頻度が高く，胎内でのサ
イトメガロウイルス感染症や1p36.3欠失症
候群，22q11.2欠失症候群などの染色体異常
のほか，*GPR56* や微小管を構成する
TUBA8，*TUBB2B*，*TUBB3* などの遺伝子
変異が同定されているが，原因不明例が多
い。遺伝子によっては常染色体劣性やX連鎖
性を示し，家族内発症がみられる。

VI．丸石様皮質異形成 cobblestone cortical dysplasia

丸石様皮質異形成は，神経細胞が皮質で停
止せずに破綻したグリア境界膜から脳表に突
出した状態である。頭部 MRI では幅の広い
脳回や不規則な脳回を呈し，厚脳回や多小脳

図6 Walker-Warburg 症候群
水頭症による頭囲拡大と著明な小脳低形成に加え，橋が前方に屈曲した特異的な所見を認める．

回と鑑別が困難な場合もあるが，国内では福山型先天性筋ジストロフィー（FCMD）の併発が多く，白質異常や小囊胞も併発し，診断は比較的容易である[8]．筋力低下に加え，70％前後の症例でてんかんを併発し，脳波では高振幅速波が特徴的である．FCMDより重症の Walker-Warburg 症候群は大脳全体の形成異常と側脳室の拡大に加え，屈曲した脳幹と小脳低形成を示し，筋ジストロフィーを伴わないこともある（図6）．

おわりに

滑脳症に代表される神経細胞移動異常症は

画像診断と遺伝子診断の組み合わせによって正確な遺伝相談が可能になる．最近では患者細胞から誘導した iPS 細胞を用いた研究や治療法開発も試みられている．滑脳症親の会 lissangel も活動し，筆者の施設では臨床と遺伝子解析に関する包括的な相談と基礎研究者との共同研究を行っており気軽に相談されたい．

文 献

1) 加藤光広：神経細胞移動障害の分子機構．日本小児科学会雑誌 111：1361～1374, 2007
2) Kato M, Dobyns WB：Lissencephaly and the molecular basis of neuronal migration. Hum Mol Genet 12 Spec No 1：R89～96, 2003
3) 加藤光広：中枢神経系の発生異常の遺伝学的背景．小児神経学の進歩 36：47～57, 2007
4) 加藤光広：大脳皮質形成異常．小児神経の画像診断 脳脊髄から頭頸部・骨軟部まで．学研メディカル，秀潤社，p.232～249, 2010
5) 加藤光広：脳形成障害・てんかんのトピックス —年齢依存性てんかん性脳症と介在ニューロン病—．脳と発達 42：333～338, 2010
6) Parrini E, Ramazzotti A, Dobyns WB et al：Periventricular heterotopia：phenotypic heterogeneity and correlation with Filamin A mutations. Brain 129：1892～1906, 2006
7) Leventer RJ, Jansen A, Pilz DT et al：Clinical and imaging heterogeneity of polymicrogyria：a study of 328 patients. Brain 133：1415～1427, 2010
8) 加藤光広：神経系の発生，中枢神経系奇形，migration の異常．小児神経学．診断と治療社，東京，p.30～39, 2008

☆　　☆　　☆　　☆　　☆　　☆